Supplementary Materials

A comparative analysis of the anatomy, phenolic profile, and antioxidant capacity of *Tussilago farfara* L. vegetative organs

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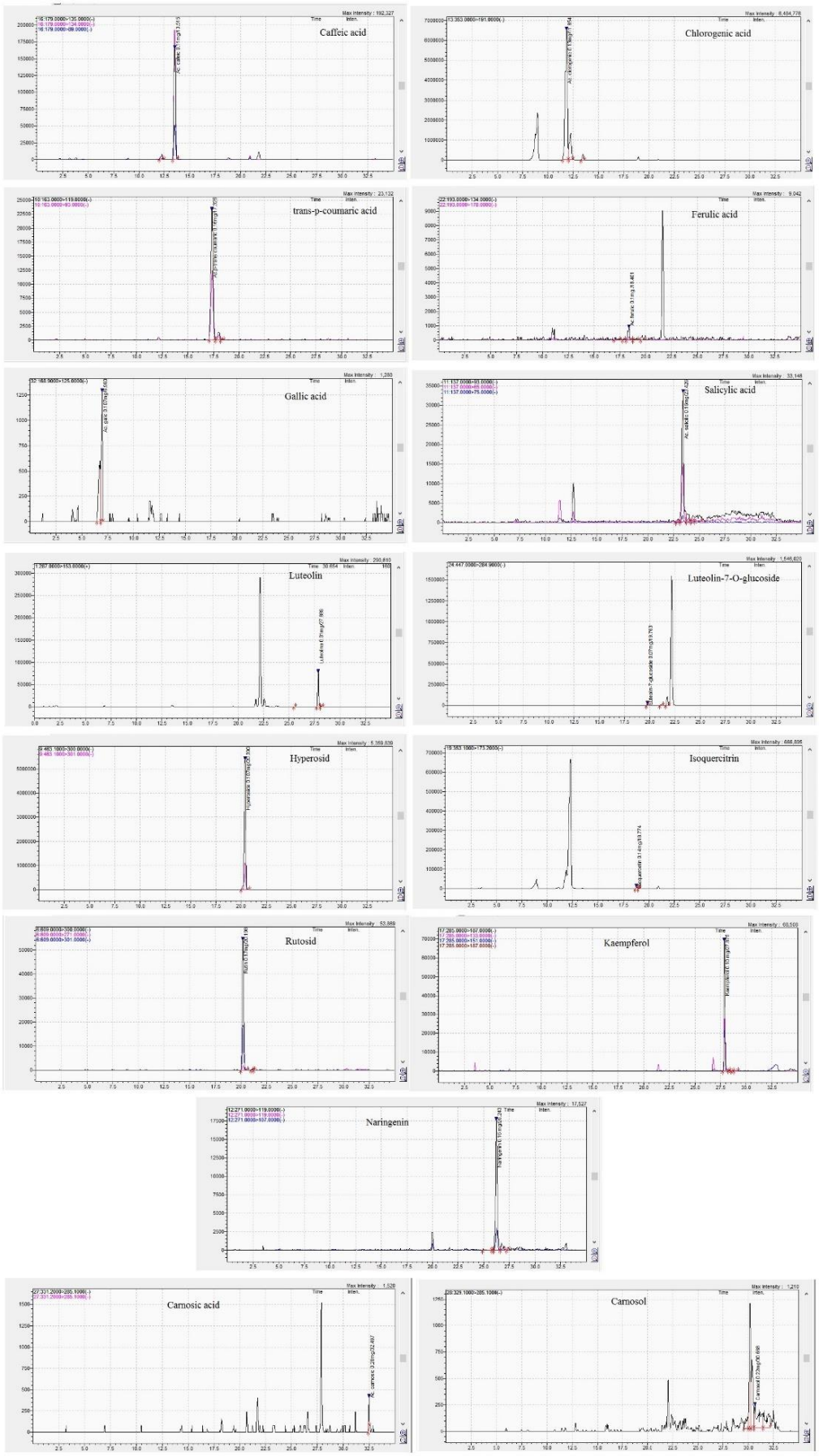

Figure S1. LC/MS chromatogram of the hydroalcoholic extract obtained from leaves of *T. farfara* L. from Rarău Mountains (RM). Compunds analysed

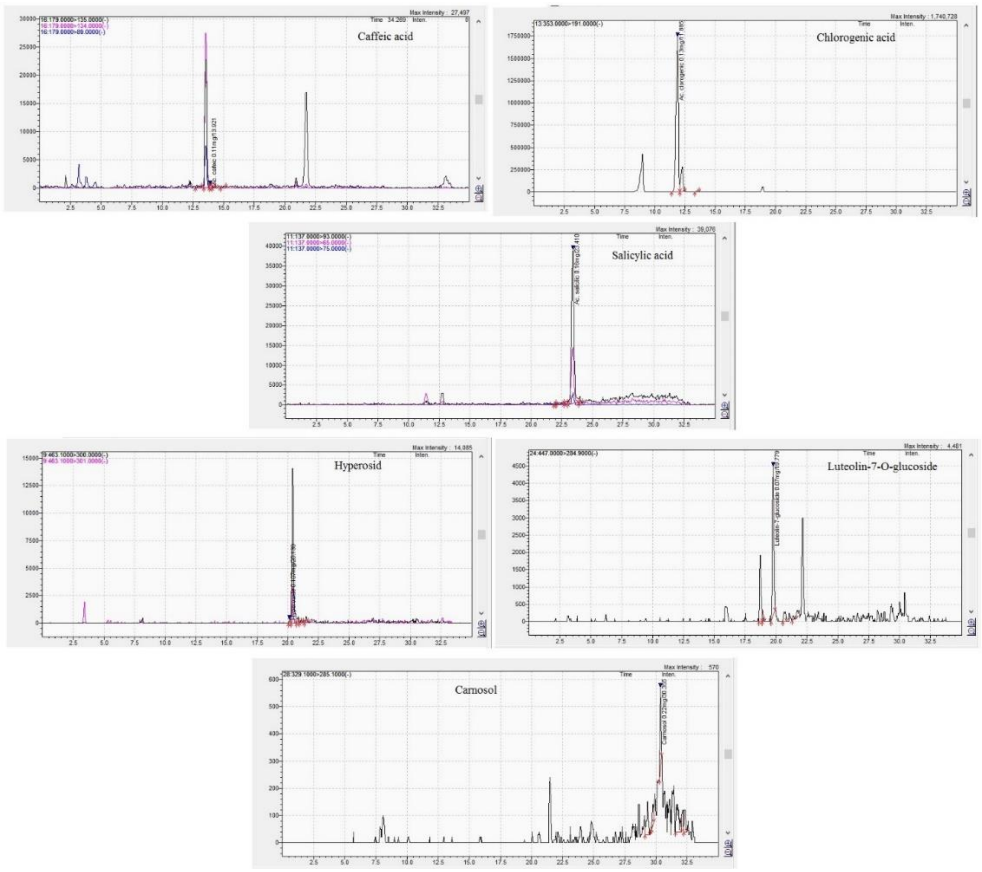

Figure S2. LC/MS chromatogram of the hydroalcoholic extract obtained from roots of *T. farfara* L. from Rarău Mountains (RM).

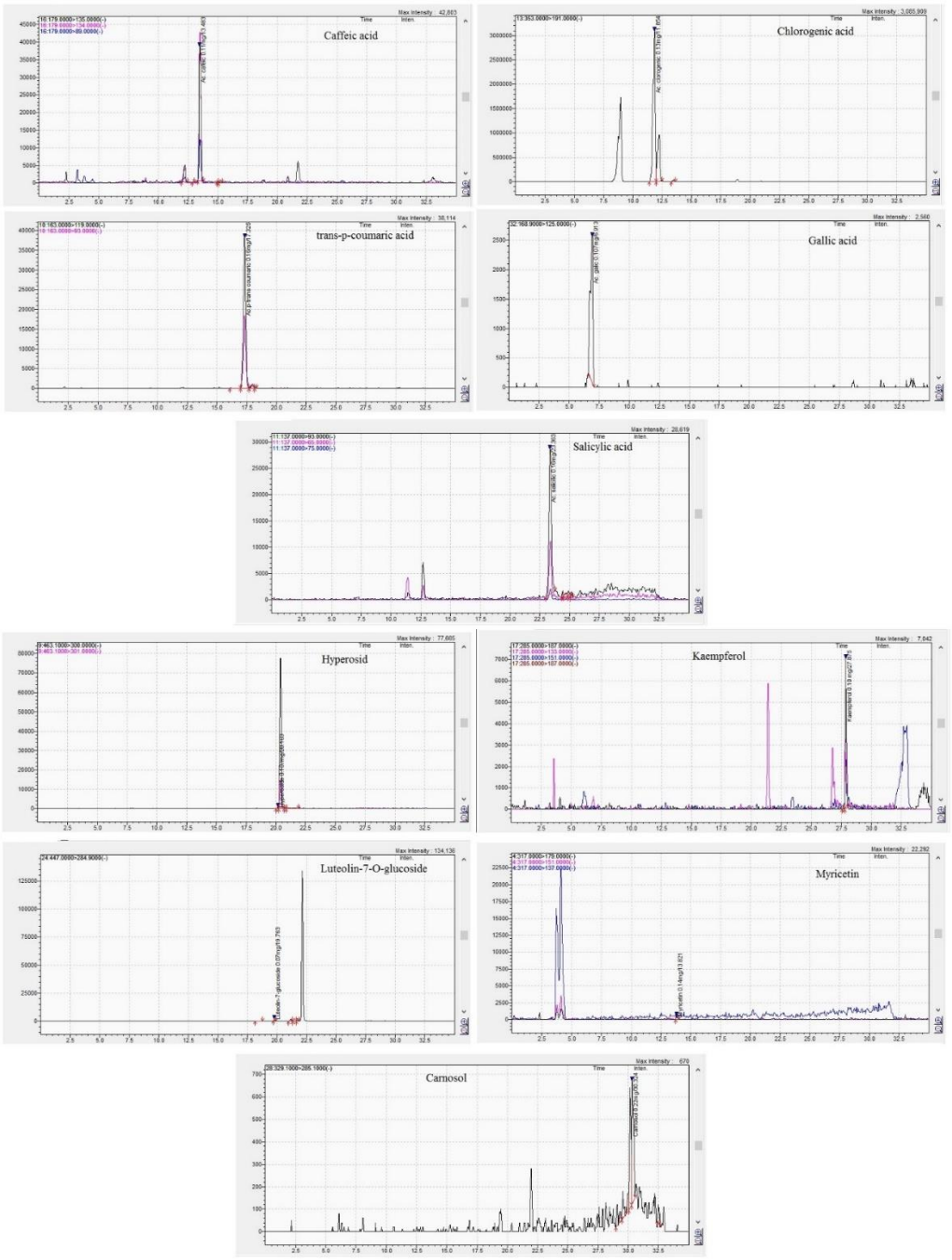

Figure S3. LC/MS chromatogram of the hydroalcoholic extract obtained from leaves of *T. farfara* L. from Vatra Dornei (VD).

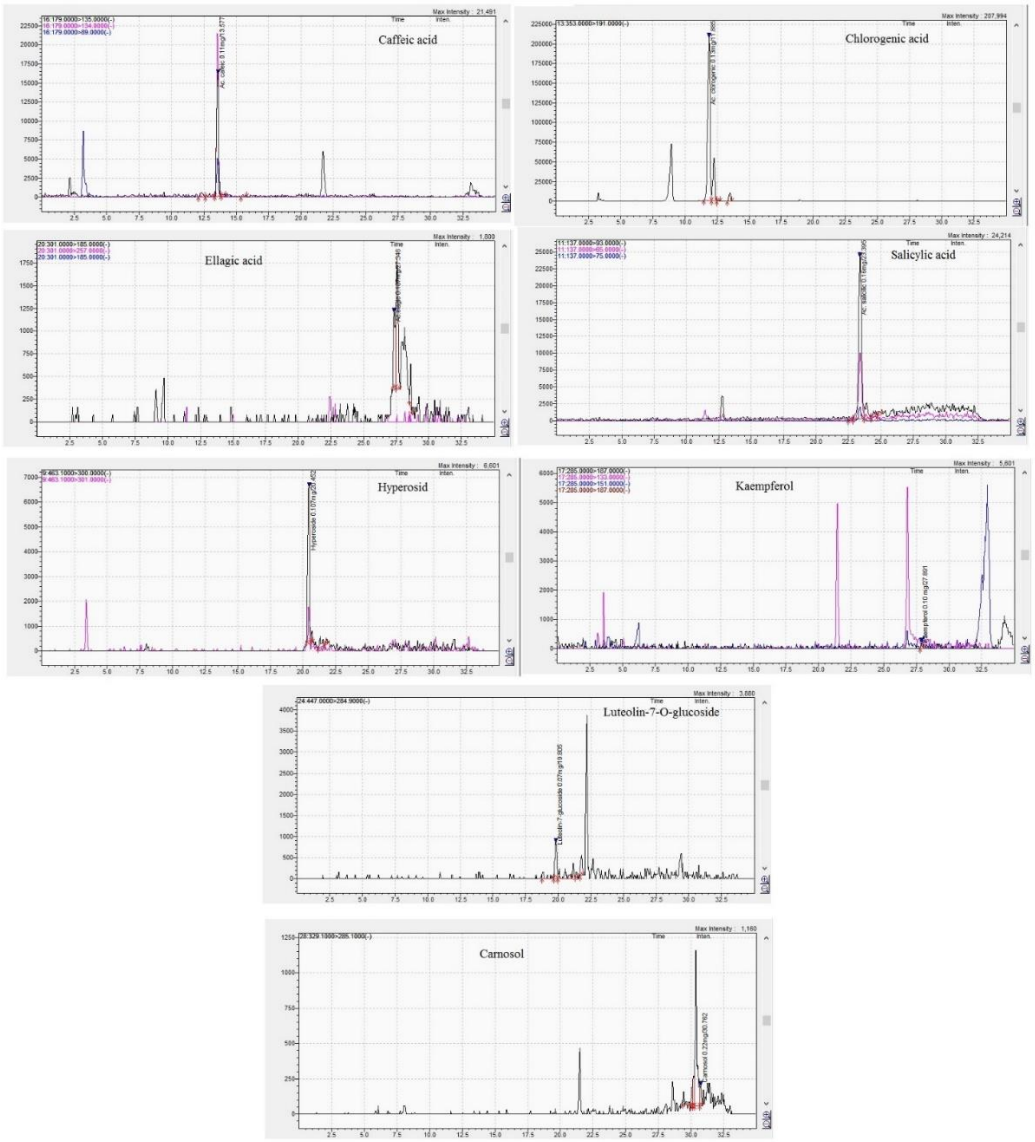

Figure S4. LC/MS chromatogram of the hydroalcoholic extract obtained from roots of *T. farfara* L. from Vatra Dornei (VD).

Table S1. Antioxidant activity of *T. farfara* extracts and isolated phytochemicals. Summarized results of original articles sorted by the plant part responsible and the extraction method.

Plant part	Preparation	Assay	Results	Ref.
Flower buds	Isolated indole alkaloids and lignans	2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay	DPPH radical scavenging activity with IC_{50} values of 45.2 ± 2.9 and 29.2 ± 2.0 μM	[35]
	Ethyl acetate fraction	Lipid peroxidation, DPPH assay,	Inhibition of lipid peroxidation by Fe^{2+} and ascorbic acid in rat brain homogenates (IC_{50} = 6.3 mg/mL), DPPH scavenging activity 50 % at 14.3mg/mL	[36]
	Ethylacetate soluble fraction, isolated compounds	Nitroblue tetrazolium (NBT) assay	Ethyl acetate extract IC_{50} =1.8 μg/mL quercetin-3-*O*-β-L-arabinopyranoside - IC_{50} = 12.9 μM; quercetin-3-*O*-β-D-glucopyranoside - IC_{50} =35.6 μM	[37]

	Polusaccharides, water-soluble crude polysaccharides	Flow Injection Analysis paired with chemiluminescence (FIA-CL)	IC_{50} ascorbic acid < IC_{50} polysaccharides < IC_{50} crude polysaccharides	[38]
	Isolated compounds	Nitric oxide inhibition assay, lipopolysaccharide-treated RAW 264.7 cells	Nitric oxide inhibitory activity ($3.5\ \mu M \leq IC_{50} \leq 28.5\ \mu M$)	[39]
	Isolated compounds	Nitric oxide inhibition assay, lipopolysaccharide-treated RAW 264.7 cells	Dose-dependend nitric oxide inhibitory activity, from $IC_{50} = 10.80\ \mu M$	[40]
	Isolated compound	Nitric oxide inhibition assay, lipopolysaccharide activated macrophages	Nitric oxide synthesis inhibition by bisabolene epoxide compound	[41]
	Isolated sesquiterpenoids	Lipopolysaccharide stimulated RAW 264.7 cells	Nitric oxide inhibitory activity	[42]
	Isolated sesquiterpenoids	*In vitro* and *in vivo* in PC12 cells and Oxidopamine (6-OHDA)-induced mouse model of Parkinson's disease	Protective effect against hydrogen peroxide and 6-hydroxydopamine-induced cytotoxicity, upregulated heme oxygenase 1 (HO-1) through interaction with Kelch-like ECH-associated protein 1 (Keap1)	[43]
	Isolated ECN sesquiterpenoid	*In vivo* study on partial sciatic nerve ligation induced peripheral neuropathic pain in albino mice	Prevention of suppresion of antioxidants such as glutathione, glutathione-S-transferase, catalase, superoxide dismutase, nuclear factor erythroid 2-related factor 2 (Nrf2), hemeoxygenase-1 and NAD(P)H-dependent quinone oxidoreductase	[44]
Leaves and flowers	Methanol extract	DPPH	Leaves antioxidant activty $IC_{50} = 55\ \mu g/mL$	[14]
Leaves	Aqueous and hydro-ethanolic extracts	ABTS, DPPH	Aqueous [µmol Trolox equivalent/g dry weight]: 320.80 (ABTS), 51.63 (DPPH); hydro-ethanolic [µmol Trolox equivalent/g dry weight]: 390.00D±1.13 (ABTS), 268.52D±1.06 (DPPH)	[12]
	Ethyl acetate (EtOAc), methanol, water (MeOH)	ABTS, FRAP, CUPRAC, phosphomolybdenum, metal chelating assays	ABTS [mg Trolox equivalent/g dry weight]: EtOAc - 41.08; MeOH - 410.98; Water - 399.18	[45]
			FRAP [mg TE/g extract dry weight]: EtOAc - 49.98; MeOH - 465.31; Water - 380.25	
			CUPRAC [mg TE/g extract dry weight]: EtOAc - 93.78; MeOH - 677.09; Water - 53.38	
			Phosphomolybdenum [mg Trolox equivalent/g extract DW]: EtOAc - 1.54; MeOH - 2.26; Water - 1.44	
			Metal chelating [mg EDTA equivalent/g dry weight]: EtOAc - 13.11; MeOH - 6.16; Water - 4.98	
	Methanol extract	DPPH	$EC_{50} = 14.54 \pm 0.72\ \mu g/mL$	[46]

	Ethanol extract	GC-MS with galvinoxyl free radical method	80-100% inhibition, highest radical scavenging efficiency out of 8 tested species	[47]
Leaves and roots	Acetone, methanol and ethanol extracts	DPPH, ABTS, FRAP	Leaves showed higher antioxidant activity than roots, and Lithuanian samples higher than French samples	[48]
Unspecified	Water and ethanol extracts	DPPH, yeast model	Water - 198.9 ± 0.71 μM ascorbate equivalent/g (DPPH); Ethanol -113.5 ± 0.00 μM ascorbate equivalent/g (DPPH); 20.9% inhibition of yeast oxidation by H_2O_2	[49]
	Methanol-water (8:2 v/v) extract	ABTS, FRAP	217.62 ± 5.35 μmol Trolox equvalent/g dry weight (TEAC), 455.64 ± 5.03 μmol Fe^{2+}/g dry weight (FRAP)	[50]

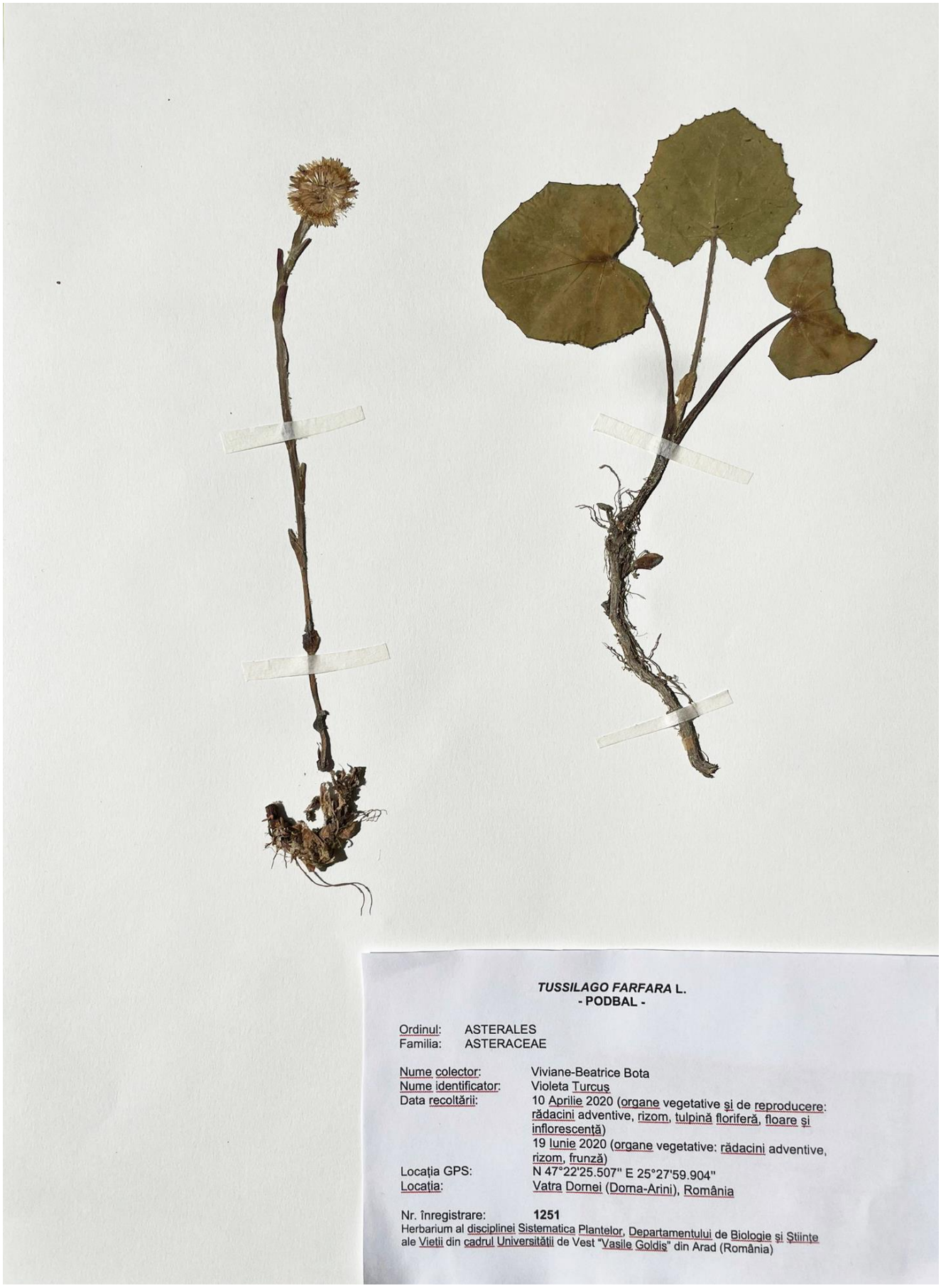

Figure S5. *Tussilago farfara* L. collected from Vatra Dornei. Registered with the voucher number 1251 in the Herbarium of the Plant Systematics discipline, part of the Department of Biology and Life Sciences of the "Vasile Goldiș" Western University of Arad (Romania).

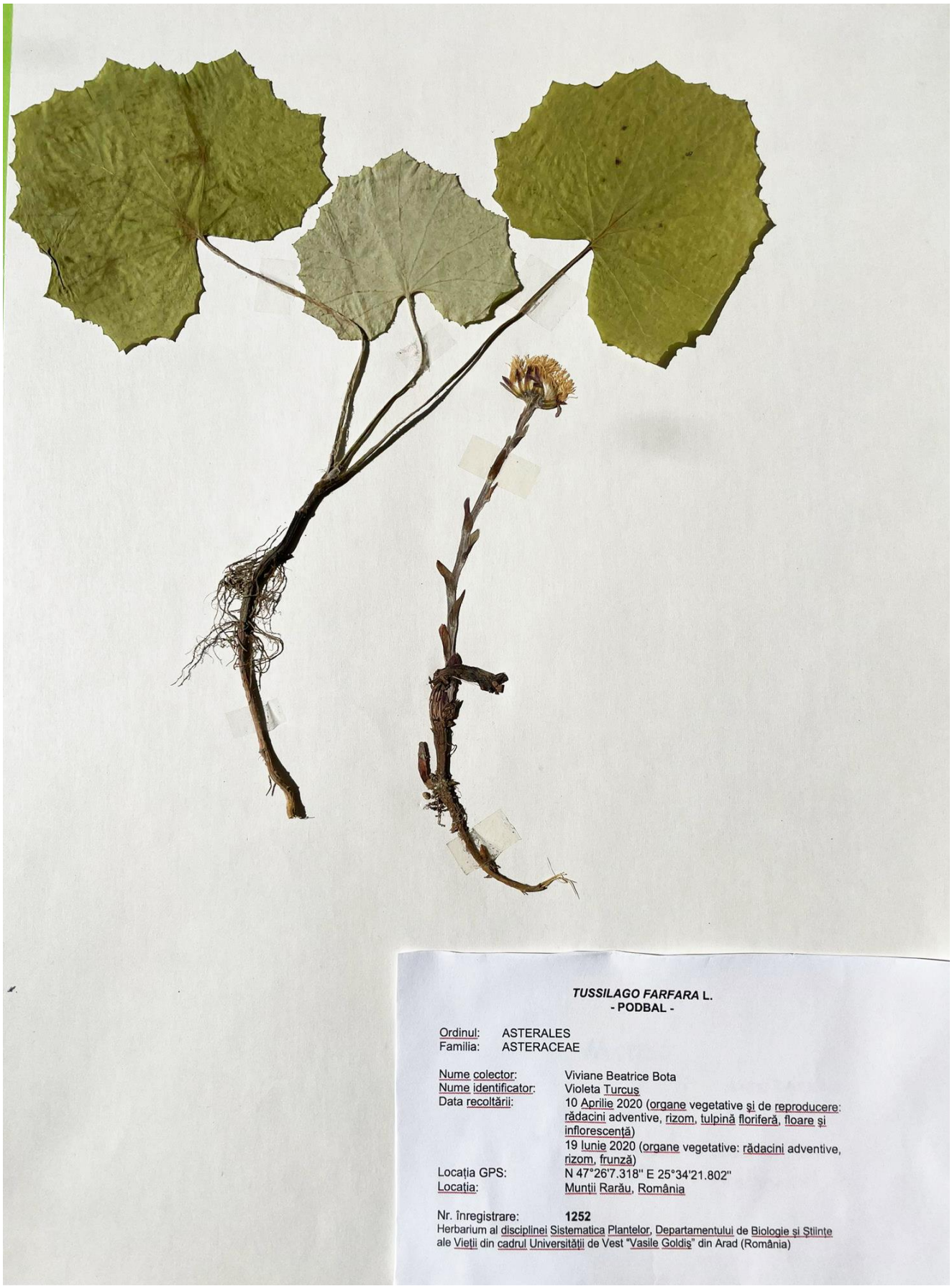

Figure S6. *Tussilago farfara* L. collected from Rarău Mountains. Registered with the voucher number 1252 in the Herbarium of the Plant Systematics discipline, part of the Department of Biology and Life Sciences of the "Vasile Goldiș" Western University of Arad (Romania).

Table S2. The standards for chromatographic and spectral data.

Standard	Retention time, min	m/z – main transition	MRM
Caffeic acid	13.8	179.0>135.0	Negative
***trans-p*-coumaric acid**	17.5	163.0>119.0	Negative
Salicylic acid	23.5	137.0>93.0	Negative
Chlorogenic acid	12.0	353.0>191.0	Negative
Apigenin	28.2	269.0>117.0	Negative
Chrysin	29.7	253.0>143.0	Negative
Luteolin	26.9	287.0>153.0	Positive
Luteolin-*7-O*-glucoside	19.9	447.0>284.9	Negative
Quercetin	25.7	300.9>151.0	Negative
Rutoside	20.3	609.0>300.0	Negative
Naringenin	26.3	271.0>119.0	Negative
Carnosic acid	32.0	331.2>285.1	Negative
Ellagic acid	27.2	301.0>185.0	Negative
Carnosol	30.7	329.1>285.1	Negative
Kaempferol	28.0	285.0>187.0	Negative
Myricetin	13.6	317.0>179.0	Negative
Hyperoside	20.3	463.1>300.0	Negative
Isoquercitrin	17.9	353.1>173.2	Negative
Ferulic acid	18.4	193.0>134.0	Negative
Gallic acid	7.0	168.9>125.0	Negative

Table S3. The standards used for LC/MS analyses.

Standard	Origin	Concentration range [mg/mL]	Calibration curve equation	Correlation factor	Detection limit [µg/mL]	Quantification limit [µg/mL]
Caffeic acid	Phytolab, Vestenbergsgreuth, Germany	0.11 – 1.10	Area = $4*10^7$*conc[mg/mL] - 319689	0.9998	3.20	4.80
***trans-p*-coumaric acid**	Phytolab, Vestenbergsgreuth, Germany	0.16 – 1.60	Area = $3*10^7$*conc[mg/mL] + 291065	0.9993	1.90	3.90
Salicylic acid	Merck, Darmstadt, Germany	0.16 – 1.60	Area = $4*10^7$*conc[mg/mL] + 44120	0.9997	1.50	2.00
Chlorogenic acid	Phytolab, Vestenbergsgreuth, Germany	0.13 – 1.30	Area = $2*10^8$*conc[mg/mL] - 269699	0.9997	5.00	8.00

Apigenin	Phytolab, Vestenbergsgreuth, Germany	0.10 – 0.98	Area = $2*10^{8}$*conc[mg/mL] + 15916	0.9999	0.20	0.30
Chrysin	Merck, Darmstadt, Germany	0.10 – 1.00	Area = $1*10^{8}$*conc[mg/mL] – 82818	0.9997	3.00	5.00
Luteolin	Phytolab, Vestenbergsgreuth, Germany	0.01 – 0.10	Area = $2*10^{8}$*conc[mg/mL] – 2295.4	0.9977	0.05	0.07
Luteolin-7-*O*-glucoside	Phytolab, Vestenbergsgreuth, Germany	0.07 – 0.70	Area = $1*10^{9}$*conc[mg/mL] – 700317	0.9990	3.00	4.00
Quercetin	Phytolab, Vestenbergsgreuth, Germany	0.09 – 0.91	Area = $5*10^{7}$*conc[mg/mL] – 9556	0.9964	0.80	1.10
Rutoside	Phytolab, Vestenbergsgreuth, Germany	0.17 – 1.70	Area = $2*10^{8}$*conc[mg/mL] – 191937	0.9996	4.00	6.00
Naringenin	Phytolab, Vestenbergsgreuth, Germany	0.16 – 1.60	Area = $3*10^{8}$*conc[mg/mL] – 43443	0.9999	0.60	0.90
Carnosic acid	Phytolab, Vestenbergsgreuth, Germany	0.28 – 2.80	Area = 10^{7}*conc[mg/mL] – 99360	0.9994	4.00	6.00
Ellagic acid	Phytolab, Vestenbergsgreuth, Germany	0.107 – 1.070	Area = 14987*conc[mg/mL] – 138.52	0.9982	3.70	5.50
Carnosol	Phytolab, Vestenbergsgreuth, Germany	0.022-0.220	Area = 10^{9}*conc[mg/mL] – 253279	0.9997	1.00	2.00
Kaempferol	Phytolab, Vestenbergsgreuth, Germany	0.10 – 1.00	Area = 10^{7}*conc[mg/mL] – 20574	0.9996	0.80	1.20
Myricetin	Phytolab, Vestenbergsgreuth, Germany	0.140 – 1.400	Area = 26499*conc[mg/mL] – 41.803	0.9997	0.60	0.90
Hyperoside	Phytolab, Vestenbergsgreuth, Germany	0.012 – 0.107	Area = $4*10^{8}$*conc[mg/mL] – 567182	0.9986	0.60	0.90
Isoquercitrin	Phytolab, Vestenbergsgreuth, Germany	0.140 – 1.400	Area = 4727*conc[mg/mL] + 68.172	0.9973	2.90	5.80

Ferulic acid	Phytolab, Vestenbergsgreuth, Germany	0.100-1.000	Area = $5*10^6$*conc[mg/mL] – 50000	0.9992	4.00	6.00
Gallic acid	Phytolab, Vestenbergsgreuth, Germany	0.107-1.070	Area = $8*10^6$*conc[mg/mL] – 37131	0.9999	1.90	2.80

Table S4. The spectral calibration curves data.

	Calibration curve equation	**Correlation factor**
Total flavonoids expressed in rutoside	Absorption = 0.024*conc[µg/mL] + 0.0031	0.9970
Total phenolic acids expressed in caffeic acid	Absorption = 0.0581*conc[µg/mL] + 0.1473	0.9855
Trolox / FRAP	Absorption = 6.0343 *conc[µM/g] + 0.0186	0.9920
Trolox / CUPRAC	Absorption = 3.7061*conc[µM/g] + 0.0093	0.9738